

May 29, 2003

**VETERINARY SERVICES MEMORANDUM NO. 800.104**

Subject: *In Vitro* Serial Release Potency Test for Completed Product  
Containing *Clostridium chauvoei*

To: Biologics Licensees, Permittees, and Applicants  
Directors, Center for Veterinary Biologics

**I. PURPOSE**

The purpose of this memorandum is to provide guidance to licensees, permittees, and applicants concerning the Center for Veterinary Biologics (CVB) policy for obtaining an exemption to use an *in vitro* potency test in place of the current Standard Requirement (SR) test for releasing serials of product containing *Clostridium chauvoei* antigen.

**II. BACKGROUND**

Currently, completed product containing *Clostridium chauvoei* antigen must be tested prior to serial release using a guinea pig vaccination-challenge potency test specified in Title 9, Code of Federal Regulations (9 CFR) Part 113.106. This test has been targeted by the CVB for replacement as part of the CVB's ongoing commitment to refine, reduce, and replace animal testing.

The Center for Veterinary Biologics Policy, Evaluation, and Licensing (CVB-PEL) has developed an *in vitro* test for quantifying *C. chauvoei* antigen in completed product, which may be used as a serial release potency test for products containing inactivated *C. chauvoei*. This test is an antibody sandwich enzyme-linked immunosorbent assay (ELISA) that uses *C. chauvoei* flagella-specific monoclonal and polyclonal antibodies to capture and detect protective flagellar antigen. The amount of flagellar antigen in the test serial is compared to that in an approved Reference Preparation. Supplemental Assay Method (SAM) 220, which describes the ELISA method, is available from the CVB-PEL.

This memorandum provides guidance on how to obtain an exemption to 9 CFR 113.106 and replace the guinea pig vaccination-challenge test with the flagellar antigen quantification test.

**III. POLICY**

Under 9 CFR 113.4, the licensee or permittee must request an exemption to the applicable SR test. Requests for exemption from 9 CFR 113.106 should contain the following:

### A. Test Validation Data

The ELISA must be validated for use with the specific products that will be tested by it.

1. *Specificity data*: Provide data that demonstrate that the antibodies used in the ELISA specifically react with the Master Seed strains of *C. chauvoei* used in the test product(s) and do not react with any other product component, including bacterial antigens, growth media, or adjuvant. Specificity data should be generated by testing sham products, made in accordance with the Outline of Production except that no *C. chauvoei* antigen is added.

2. *Reproducibility data*: Demonstrate that the test, when conducted by the licensee or permittee, produces reproducible results. At least three serials of each product that will be tested for potency by the ELISA should be assayed. Each serial should be tested in six independent replicate assays. Assays should be performed by a minimum of two different individuals. Data from a minimum of 18 tests (3 replicates of 3 serials by each of 2 technicians) per product should be submitted.

3. *Dose-response data*: Demonstrate that the ELISA is capable of discriminating between products formulated with *C. chauvoei* antigen content  $\pm 10\%$  and  $\pm 20\%$  of the proposed release value.

4. *Correlation data*: Demonstrate that the ELISA correctly identifies satisfactory and unsatisfactory serials when compared to the SR guinea pig vaccination-challenge test. Data generated by testing diluted products used for reference qualification (see Section III.C.2 below) may be submitted as part of the test correlation data.

### B. Changes to the Outline of Production

1. *Test Method*: SAM 220 must be cited, or an acceptable alternative ELISA procedure for quantifying the flagellar antigen of *C. chauvoei* must be described, in Section V.C of the Outline of Production or a Special Outline.

2. *Information regarding Reference Preparations*: The identity and expiration date of the approved Reference Preparation must be included in Section V.C of the Outline of Production.

3. *Test Criteria*: The requirements for a satisfactory test, along with validity requirements, must be included in Section V.C of the Outline of Production. If specified in Section V.C of the Outline of Production, the guinea pig vaccination-challenge test described in the SR may be used as a second-stage test on serials failing the ELISA potency test.

4. *Date of Exemption*: The date that the exemption to the SR test was approved by APHIS must be recorded in Section V.C of the Outline of Production.

### C. Qualification of Reference Preparation

1. *Initial Qualification*: For products that have acceptable host animal challenge data on file, Reference Preparations may be qualified based on serological titer in cattle, provided that the humoral response to vaccination meets a minimum standard (see below). Products for which there are no acceptable host animal challenge data on file, and those candidate Reference Preparations failing to induce an adequate serological titer, must be proven efficacious by a vaccination-challenge study performed in cattle. Qualifying serials for all Reference Preparations must be made according to a filed Outline of Production. Reference Preparations made using antigen levels lower than those specified in a filed Outline of Production may not be qualified solely by serology. References with a larger number of components may be used for smaller combination products provided that the adjuvant is identical and the relative potency passing requirement is 1.0.

The reference qualification study should include at least 10 vaccinates and 5 control animals. Cattle used in the study must have a *C. chauvoei* agglutination titer <10 prior to vaccination. The cattle must be vaccinated according to the label directions. Two weeks after vaccination, *C. chauvoei* agglutination titers are measured; agglutination tests must be conducted using standardized antigen and control sera obtained from the CVB-PEL. If at least 8 of 10 of the vaccinated cattle demonstrate agglutination titers =100, the Reference Preparation is acceptable based on serology. If fewer than 8 vaccinated cattle achieve agglutination titers =100, all cattle may be challenged with a virulent *C. chauvoei* spore suspension. If at least 8 of 10 of the vaccinated cattle survive the challenge, and at least 4 of 5 of the control cattle die as a result of the challenge, the Reference Preparation is satisfactory.

2. *Laboratory Animal Model*: It is highly recommended that all Reference Preparations be evaluated in guinea pigs concurrently with the initial qualification study in cattle. Using the vaccination/challenge methods outlined in SAM 200, Supplemental Assay Method for Potency Testing Products Containing *Clostridium chauvoei* Antigen, establish the 50% protective dose (PD50) of the Reference Preparation in guinea pigs. Vaccinate groups of five guinea pigs with fractional doses of the Reference Preparation, using serial dilutions no greater than two-fold. Repeat the PD50 determination in at least four independent assays. The guinea pig PD<sub>50</sub>, once established, may be used as a standard to requalify existing approved Reference Preparations and to qualify new Reference Preparations.

3. *Confirmation by Center for Veterinary Biologics-Policy, Evaluation, and Licensing (CVB-PEL)*: All candidate Reference Preparations will be subject to CVB-PEL confirmatory testing by 9 CFR 113.106 before final APHIS approval is granted.

D. Applicability of Exemption

The testing exemption will apply only to the serial release tests conducted by licensees and permittees. For a transition period of 5 years, serials will continue to be subject to CVB-PEL confirmatory testing by 9 CFR 113.106. The disposition of all serials tested by CVB-PEL will be based solely on the 9 CFR 113.106 potency test result. This transition period will be used for ongoing quality assurance monitoring. At the end of the transition period, if the cumulative data confirm that the correlation between the guinea pig and ELISA tests is acceptable under widespread use in typical manufacturing settings, CVB will take the appropriate steps to codify the *in vitro* method as the SR test in place of the guinea pig vaccination-challenge test.

/s/ W. Ron DeHaven

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